

# A STUDY OF THE SIZES OF *ENTAMOEBIA HISTOLYTICA* CYSTS AMONGST SYMPTOMLESS CARRIERS IN JAMAICA

BY

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(Received for publication 13 April, 1921)

This investigation was carried out coincidentally with another which was undertaken to determine the incidence of intestinal parasites amongst patients in hospital for some cause other than dysentery or other intestinal complaints. Of the various patients whose stools were examined, only those with a considerable number of cysts will be dealt with in detail; others will receive briefer mention but will be included in the total of all the cysts measured.

Malins Smith (1918) has shown that the curve obtained by measurement of one thousand cysts of *Entamoeba histolytica* from thirty cases is bimodal, between  $7\mu$  and  $8\mu$  and between  $12\mu$  and  $13\mu$ ; while that for *Entamoeba coli* is unimodal, between  $16\mu$  and  $17\mu$ . He also found that cases with cysts averaging between  $9\mu$  and  $10\mu$  were very rare. He demonstrated that the two chief strains were the 'small', averaging  $7.7\mu$ , and the 'ordinary', averaging  $12.6\mu$ , and that in England infections with the former were rare in those who had never been out of the country.

It was his experience also that each strain persisted for a long time if left without treatment, or without being effectively treated, and that the strains did not replace each other.

Dobell and Jepps (1918) claim to have established that there are five strains of *Entamoeba histolytica* cysts, those occurring most frequently being approximately  $6.6\mu$ ,  $8.3\mu$ ,  $11.6\mu$ ,  $13.3\mu$  and  $15\mu$ .

'For the complete demonstration of the existence of strains in cysts of *Entamoeba histolytica* it is necessary to prove that the mean diameter of cysts from any patient is not subject to any considerable variation from day to day, but remains constant.'

As regards the question as to whether change in size of cysts in *Entamoeba coli* can occur from day to day, or rather from one day to another, there is not sufficient evidence at hand, according to Malins Smith.

In another paper by the same author (1919), he states that samples of one hundred cysts (he is speaking of *Entamoeba histolytica*), taken on different days, often differ much more in average size than samples from the same stool, so that these differences cannot be accounted for by errors in sampling, and it has been suggested by Dobell and Jepps (*loc. cit.*) that emetine treatment may affect the size of the cysts, in that if they appear after treatment they are liable to be larger than on normal occasions. In the series dealt with below this question of the effect of emetine does not come into discussion at all, since none of the cases showed any intestinal symptoms to warrant interference, and they were not given any treatment for the eradication of the cysts.

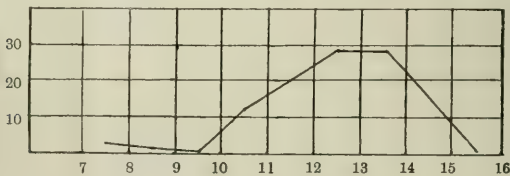
When mixtures of 'small' and 'ordinary' strains are found it is only natural to expect that the cysts may appear in different proportions on different days, and thus there will be differences in the average size of the cysts.

The criteria relied upon for differentiation of the cysts were those stated in a previous paper on the incidence of intestinal parasites in symptomless carriers in Jamaica, namely, the size and shape of the cyst, the characters of the cyst-wall, the number of nuclei and arrangement of the nuclear chromatin, the cytoplasm and its inclusions—chromatoid bodies and vacuoles.

One of the most interesting of the cases in which cyst measurements were made regularly and in considerable numbers was that of

I. E. H., male. At his first examination cysts of both *Entamoeba coli* and *E. histolytica* were found in fairly large

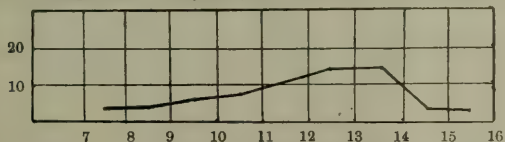
FIG. 1. Showing size of *E. histolytica* cysts (72). Case I. First examination.



numbers; one hundred and thirty-two *coli* and seventy-two *histolytica* were measured. To save a long description, the curve (following the method of Matthews and Malins Smith) below gives at a glance the relative numbers present of cysts of the different sizes. To avoid making the curve too complicated, the abscissae are drawn at intervals representing  $1\mu$ , and the ordinates at intervals representing 10 units.

At the second examination, three days later, the numbers of cysts found were less, but still considerable; the *histolytica*, however, were rather more often met with, in the proportion of six to five, whereas in the previous examination the *coli* were nearly twice as numerous as the *histolytica* (this may, of course, be a mere sampling coincidence). As regards the *coli* cysts, there is not much difference to notice, except that those most frequently met with on the first day were between  $16\mu$  and  $17\mu$ , constituting nearly 50 per cent. (sixty-one out of one hundred and thirty-four), whereas on the next occasion thirteen out of fifty were between  $17\mu$  and  $18\mu$ . As regards the *histolytica* cysts, those most frequently met with, and those nearest approaching them, were practically the same as on the first occasion.

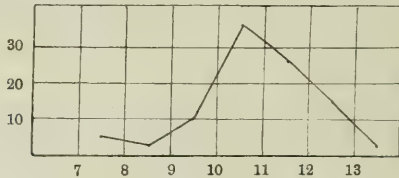
FIG. 2. Showing size of *E. histolytica* cysts (60). Case I. Second examination.



This patient then left hospital for a week to see after some private matters at home; further examinations were carried out on his return. Not only were *coli* cysts quite infrequently met with, but *histolytica* were numerous; in the course of measuring one hundred of the latter, only twenty of the former were encountered. More than one-third (seven) of the *coli* cysts were  $19.4\mu$  in diameter, and the average size of all was  $18.64\mu$ ; only one was found of the size ( $16\mu$  to  $17\mu$ ) which was most frequent at the first examination. Also, as the curve below shows, the *histolytica* cysts were distinctly smaller, though still nearer the size of the 'ordinary' strain than the 'small'. Thus, at the first examination the most frequent were

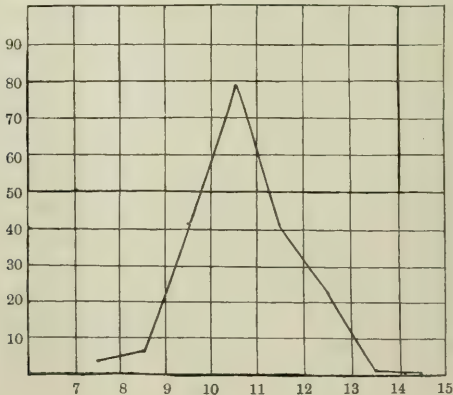
12.6 $\mu$  and 13.2 $\mu$ , while they were now at the third examination 10.6 $\mu$  and 11.2 $\mu$ .

FIG. 3. Showing size of *E. histolytica* cysts (100). Case I. Third examination.



Two days later a fourth examination was made of the stools from this patient, and again *histolytica* cysts were found in fair numbers, while *coli* cysts were conspicuous by their absence. While measuring two hundred of the former, only five of the latter were encountered. Though among the *histolytica* cysts the size most frequently met with was that of diameter 10.6 $\mu$ , as before, nevertheless, as the curve shows, those a little smaller still were present in considerably greater proportion than on previous occasions. It is a matter of much regret that this patient left hospital just afterwards, so that I was not able to obtain further specimens, for though, as already stated, the strains do not replace each other, nevertheless, these

FIG. 4. Showing size of *E. histolytica* cysts (200). Case I. Fourth examination.



findings are at variance with the statement that 'the mean diameter of cysts from any patient is not subject to any considerable variation from day to day, but remains constant.'

Thus, the sizes  $12.6\mu$  and  $13.2\mu$  most frequent at the first examination became at the third examination, a week or ten days later,  $10.6\mu$  and  $11.2\mu$ ; and cysts  $13.2\mu$  in size, the numbers of which (viz., twenty-seven and fifteen) were only one less than the most frequent numbers (viz., twenty-eight and sixteen) in the first and second specimens examined, had dropped at the fourth examination to only 1.5 per cent. of the cysts found.

Fig. 5, in which the curves for the different examinations are placed on one chart, the proportions of cysts of various sizes being reduced to percentages, shows well the change which occurred in the size of the cysts in the course of a fortnight or so.

FIG. 5. Showing size of *E. histolytica* cysts. Case I. Four examinations.

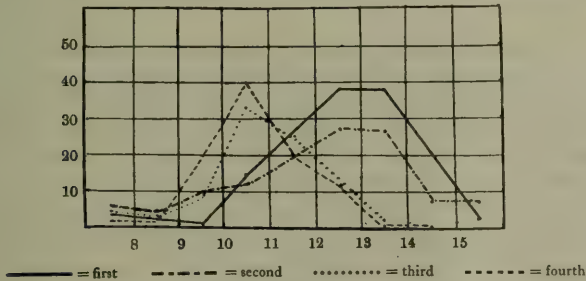
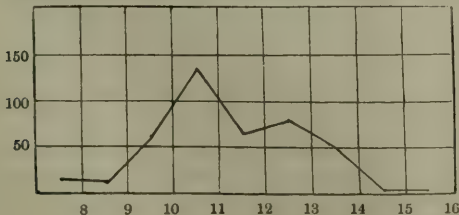


Fig. 6 gives the curve for the whole four hundred and thirty-two *histolytica* cysts measured in this case.

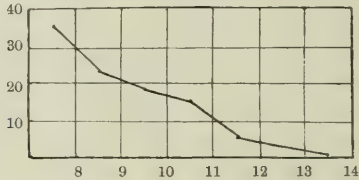
FIG. 6. Showing size of *E. histolytica* cysts (432). Case I.



II. M.W., female. Several examinations were made also of this case, and, except on one occasion, *histolytica* cysts were much more numerous than *coli* cysts. On the first occasion on which one hundred were measured, 25 per cent. were of a diameter of  $7.9\mu$ ; this was the size most frequently met with, while the mean for the one hundred was  $8.94\mu$ , and thirty-five were between  $7\mu$  and  $8\mu$  in diameter.

Fig. 7 shows the curve of distribution on this occasion.

FIG. 7. Showing size of *E. histolytica* cysts (100). Case II. First examination.



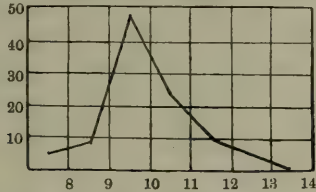
On comparing these findings with subsequent ones, the condition is most interesting, and I can find no other explanation than that either this patient was infected with both the 'small' and 'ordinary' varieties, or rather with the 'small' and one or more of the larger varieties, and that after the first occasion the 'small' failed to appear, or, secondly, that a change took place of the nature of conversion of the small into the larger. This, it is generally held, does not occur, though, as already stated, Dobell suggests that after emetine treatment, if cysts reappear, they may be larger than before treatment. In this case, as with the others, no emetine at all was given.

There is one other explanation of the findings, namely, that the wrong stool was sent up on the first day, as the findings then were so different from those on subsequent occasions, but every care was taken to avoid such a mistake. The patients were told what was wanted and the reason for it, and the nurses were also asked to take particular care to obtain the specimens fresh and send them up as soon as possible after the passing of the stool.

Moreover, on comparing the figures for the different examinations, it will be seen that the transition of the mode of the curve is gradual. Thus, in fig. 7 the greatest number had a diameter

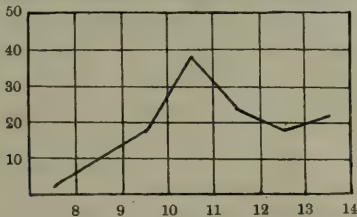
between  $7\mu$  and  $8\mu$ ; after an interval of two examinations in which no *histolytica* cysts were found at all, they appeared in large numbers, those most in evidence having a diameter between  $9\mu$  and  $10\mu$ .

FIG. 8. Showing size of *E. histolytica* cysts (100). Case II. Second examination.



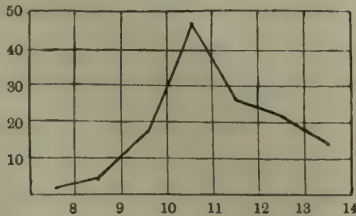
On the third occasion those most frequently encountered had an average diameter between  $10\mu$  and  $11\mu$ , and in addition there were more cysts between  $13\mu$  and  $14\mu$  than at the previous examinations.

FIG. 9. Showing size of *E. histolytica* cysts (125). Case II. Third examination.



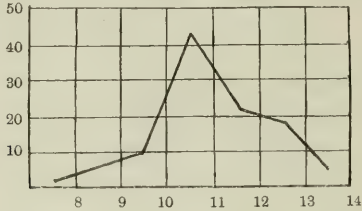
At the fourth examination, at which the cysts were numerous, those between  $10\mu$  and  $11\mu$  were again the most frequent, and even more so than at the previous examination.

FIG. 10. Showing size of *E. histolytica* cysts (130). Case II. Fourth examination.



The fifth examination showed a similar state of things as regards those between  $10\mu$  and  $11\mu$ , while those between  $13\mu$  and  $14\mu$  were becoming less again.

FIG. 11. Showing size of *E. histolytica* cysts (100). Case II. Fifth examination.



Finally, a short time before the patient left hospital the number of cysts generally was less, but though those with diameter between  $10\mu$  and  $11\mu$  comprised about 25 per cent., there was a still proportion, namely, fifteen out of forty (*i.e.*, 37 per cent.) with a diameter between  $12\mu$  and  $13\mu$ , *i.e.*, the size known as 'ordinary.'

We note, therefore, the peculiar fact that in the course of a month there was a change from cysts of the 'small' variety being most numerous to those of the 'ordinary' variety, passing through intermediate phases.

This is graphically shown in fig. 12, which gives the superposed curves of the six examinations, where the shifting of the mode to the right is very evident. It will be noted that the curves for the third, fourth and fifth counts strongly resemble one another.

FIG. 12. Showing size of *E. histolytica* cysts. Case II. Six examinations.

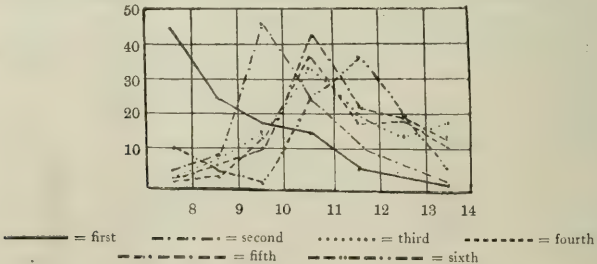
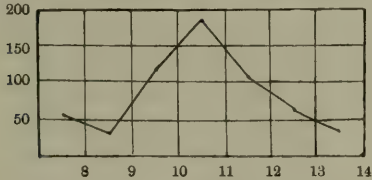




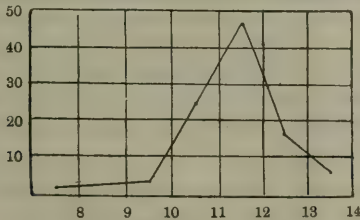
Fig. 13 gives the single curve for the whole five hundred and ninety-five cysts measured from this case.

FIG. 13. Showing size of *E. histolytica* cysts (595). Case II.



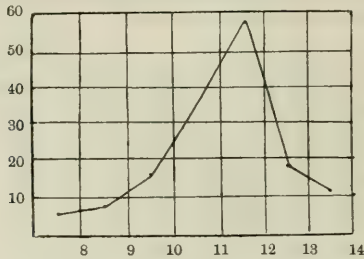
III. C. H., male. This patient's stools showed cysts of *Entamoeba histolytica* on two occasions during the three weeks, the second being five days after the first. On the first, one hundred cysts were measured, and, as the following curve shows (fig. 14), those most frequently met with had a diameter of  $11.2\mu$ , while 47 per cent. were between  $11\mu$  and  $12\mu$ , and the mean for the one hundred was  $11.32\mu$ .

FIG. 14. Showing size of *E. histolytica* cysts (100). Case III. First examination.



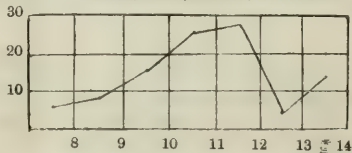
On the other occasion on which *histolytica* cysts were found, out of the fifty measured there were ten each of  $9.3\mu$  and  $11.5\mu$ , and an average diameter of  $10.2\mu$ ; the greatest number (26 per cent.) were between  $9\mu$  and  $10\mu$ .

A curve of all the *histolytica* cysts measured from this case (one hundred and fifty) is given in fig. 15.

FIG. 15. Showing size of *E. histolytica* cysts (150). Case III.

IV. T. M., male. On the first occasion on which cysts were found in specimens from this patient, *histolytica* and *Giardia* only were met with, and of the former very few—twenty only in a prolonged search. Of these between one-third and a half showed asymmetry, so that the mean of the two diameters was taken as the measurement; of the twenty there were six each between  $7\mu$  and  $8\mu$  and between  $8\mu$  and  $9\mu$ , the remaining eight being between  $11\mu$  and  $12\mu$ .

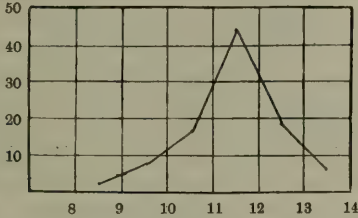
For five days after this *Giardia* only was found, and then *histolytica* was encountered in considerable numbers. One was able to measure one hundred with very little searching, and during the measuring of these a few *coli* cysts were also found, but only five of them. The distribution of sizes of the one hundred *histolytica* cysts is shown in fig. 16. As with the twenty seen on the first occasion, only six were of the small size, the greatest number being between  $11\mu$  and  $12\mu$  as before, but this only exceeded by one that of those between  $10\mu$  and  $11\mu$ .

FIG. 16. Showing size of *E. histolytica* cysts (100). Case IV. First examination.

They were not found again till four days later, when practically the same condition of things was noted and cysts were encountered

in the same relative proportion, namely, six *coli* to one hundred *histolytica*. Those met with most frequently were of the same size as before, but the mean was higher,  $11.6\mu$  as against  $10.8\mu$  on the previous occasion, a difference, possibly, not more than would be accounted for by sampling errors with so small a number as one hundred.

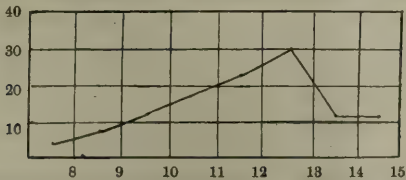
FIG. 17. Showing size of *E. histolytica* cysts (100). Case IV. Second examination.



The following five cases do not call for much individual or detailed description. In all of them *histolytica* cysts were only found occasionally, perhaps at one examination; their claim to mention consists in the fact that when they were found one hundred cysts were measured, so that a percentage of size was obtainable.

V. C. M., male. At the first examination a few *coli* cysts only were found; during the next twelve days no cysts of any kind were seen, then *coli* in small numbers. *Giardia* very numerous and one hundred *histolytica* were measured. During the succeeding twelve days again no cysts were seen in spite of repeated examinations, but then a few *coli* again made their appearance.

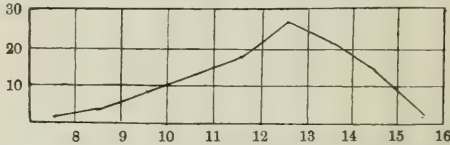
FIG. 18. Showing size of *E. histolytica* cysts (100). Case V.



VI. R. W., male. On two occasions only in three weeks were cysts found in the stools of this patient. At the first there were not

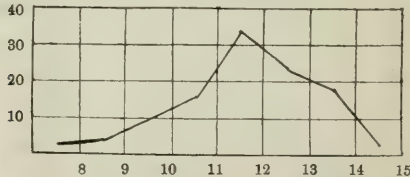
many of either form, but twenty-six *coli* were found to twelve *histolytica*. On the second occasion, eleven days later, almost the same number of *coli* were seen, but *histolytica* were more numerous, one hundred being measured. Those most frequently met with were between  $12\mu$  and  $13\mu$ , with a mean for the one hundred cysts of  $12.3\mu$ . This corresponds, therefore, to the 'ordinary' type of cyst infection.

FIG. 19. Showing size of *E. histolytica* cysts (100). Case VI.



VII. W. F., male. The occurrence of cysts in this patient was very irregular. At almost the first examination *histolytica* cysts were met with in considerable numbers, and one hundred were measured. For the next four days none were seen at all; then a few, eight only, were found after a long search. They were again absent, or perhaps it were better to say undiscovered, for nine days, and then in small numbers only and with *Giardia* cysts. Two days afterwards *coli* cysts appeared for the first time since the examination was begun, though not in large numbers, and *histolytica* was present in small numbers as before, *Giardia* being numerous. A curve is given for the occasion on which one hundred cysts were measured. Those most frequently met with had a diameter of  $11.6\mu$ , and the mean was  $11.69\mu$ .

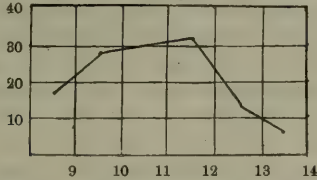
FIG. 20. Showing size of *E. histolytica* cysts (100). Case VII.



VIII. M. S., female. On four occasions *histolytica* cysts were seen in this case; the interval between two appearances was ten days in one instance. Three times out of the four a few cysts only were seen, namely, nine, six, and fifteen, but once one hundred were

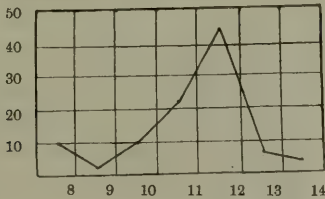
found and measured. *Coli* cysts were usually present too, but not in large numbers, and on one occasion none could be found at all.

FIG. 21. Showing size of *E. histolytica* cysts (100). Case VIII.



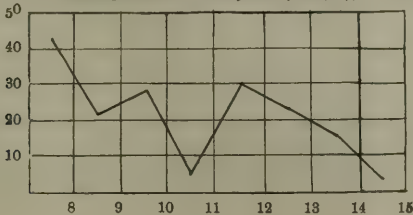
IX. M.S., female. This patient left hospital after two examinations. At one no cysts were detected at all, at the second *histolytica* cysts were found to be fairly numerous, while *coli* were present also but in smaller numbers (one to four); nearly half of the *histolytica* varied between  $11\mu$  and  $12\mu$  in diameter.

FIG. 22. Showing size of *E. histolytica* cysts (100). Case IX.



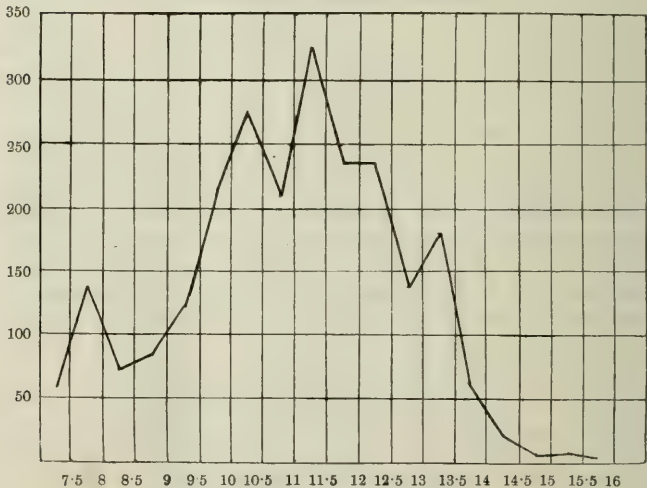
X. Finally, there may be mentioned a case in which *histolytica* cysts were found on several occasions in conjunction with *coli*, but at no time were they numerous. Fifty were measured twice, and altogether a total of one hundred and sixty-seven. Those most frequently met with had a diameter between  $7\mu$  and  $8\mu$ , and next to this between  $11\mu$  and  $12\mu$ .

FIG. 23. Showing size of *E. histolytica* cysts (167). Case X.



From three other cases between fifty and one hundred cysts were measured, while in five more a smaller number still were found. Some of these patients left hospital before the investigation could be completed, or were transferred to other wards and the stools were not sent up regularly. It would serve no useful purpose, therefore, to discuss these in any detail; the total has been included in the two thousand, three hundred and eighty-nine *histolytica* cysts measured. This number includes cysts from eighteen patients, of whom ten contributed over one hundred cysts each, three between fifty and one hundred, and the remaining five a smaller number. It may be repeated once again that none of these patients gave a history indicative of previous attack of acute dysentery (this does not preclude their having had such an attack) and none were suffering from any intestinal trouble during the time they were under investigation.

FIG. 24. Showing size of *E. histolytica* cysts (2,389).

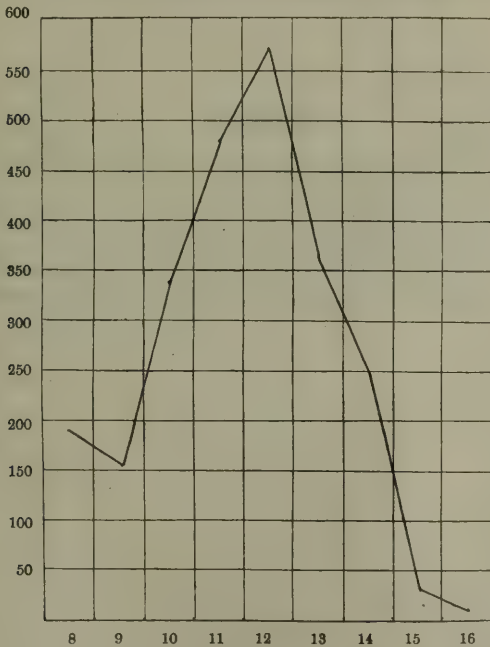


If one considers fig. 24, the curve would appear to be a multi-modal one, with a mode between  $7.5\mu$  and  $8\mu$  (corresponding to the

'small' variety) with an average diameter of  $7.7\mu$ ; a second between  $10\mu$  and  $10.5\mu$ ; a third between  $11\mu$  and  $11.5\mu$ ; a fourth about  $12\mu$ , and a fifth between  $13\mu$  and  $13.5\mu$ .

It is worth noting that there is among this series no mode at  $12.6\mu$  as has been found by Matthews and Malins Smith at home, but there is a very marked one practically corresponding to the  $11.6\mu$  variety of Dobell and Jepps, and also one corresponding to the  $13.3\mu$  variety (I forbear to call them 'strains') of the same author.

FIG. 25. Showing size of *E. histolytica* cysts (2,389).



When, however, the figure is drawn up giving the numbers found within certain intervals of a complete  $1\mu$ , the curve then appears as a bimodal one (fig. 25), though the second mode does not occur

between  $12\mu$  and  $13\mu$ , as Malins Smith found, but between  $11\mu$  and  $12\mu$ .

The interpretation of this I cannot give, for further examinations for a prolonged period of a large number of cases would be necessary before any dogmatic statement could be brought forward. Whether the prolonged infection with the cysts of *Entamoeba histolytica* leads to their becoming reduced in size, or whether the strain in Jamaica is not the same as the 'ordinary' strain found at home, but a little smaller, is mere conjecture and not worth serious consideration with the small data at present available.

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